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L6: Entry 1 of 1 File: USPT May 16, 2000

US-PAT-NO: 6063608
DOCUMENT-IDENTIFIER: US 6063608 A

TITLE: Cloned genes encoding reverse transcriptase lacking RNase H activity

DATE-ISSUED: May 16, 2000

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/194; 435/252.3, 435/252.33, 435/320.1, 435/475, 435/69.1, 435/91.1, 435/91.2, 435/975, 536/23.2

CLAIMS:

What is claimed is:

1. An isolated polypeptide having DNA polymerase activity and substantially reduced RNase H activity, wherein said polypeptide is encoded by a modified reverse transcriptase nucleotide sequence that encodes a modified amino acid sequence resulting in said polypeptide having substantially reduced RNase H activity, and wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

2. The polypeptide of claim 1, wherein said nucleotide sequence is derived from an organism selected from the group consisting of M-MLV, HTLV-1, BLV, and HIV.

3. The polypeptide of claim 1, wherein said polypeptide has no detectable RNase H activity.

4. The polypeptide of claim 1, wherein said polypeptide lacks RNase H activity.

5. The polypeptide of claim 1, wherein said polypeptide may be used in the preparation of full length cDNA.

6. The polypeptide of claim 5, wherein said preparation of full length cDNA may be accomplished without significant degradation of an mRNA template.

7. The polypeptide of claim 1, wherein said polypeptide allows the preparation of cDNA without significant degradation of an mRNA template.

8. The polypeptide of claim 1, wherein said polypeptide allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

9. The polypeptide of claim 1, wherein said polypeptide does not significantly degrade an mRNA template during first strand DNA synthesis.

10. The polypeptide of claim 1, wherein said polypeptide does not significantly degrade an mRNA template during a cDNA synthesis reaction as shown in FIG. 5.

11. The polypeptide of claim 1, wherein said polypeptide does not significantly degrade

an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

12. The polypeptide of claim 1, wherein said polypeptide allows an mRNA template to remain intact during cDNA synthesis as shown in FIG. 5.

13. The polypeptide of claim 1, wherein said polypeptide allows an mRNA template to remain intact during a one minute cDNA synthesis reaction as shown in FIG. 5.

14. The polypeptide of claim 1, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during cDNA synthesis.

15. The polypeptide of claim 1, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template by gel electrophoresis as shown in FIG. 5.

16. The polypeptide of claim 1, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

17. The polypeptide of claim 1, wherein said polypeptide can synthesize cDNA from an mRNA template.

18. The polypeptide of claim 17, wherein said polypeptide can synthesize full length cDNA.

19. The polypeptide of claim 17, wherein said polypeptide can synthesize cDNA without significant degradation of said mRNA template.

20. The polypeptide of claim 1, wherein said nucleotide sequence is derived from a Moloney Murine Leukemia virus (M-MLV) nucleotide sequence.

21. The polypeptide of claim 1, wherein said nucleotide sequence is derived from a retrovirus nucleotide sequence.

22. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a modified reverse transcriptase nucleotide sequence that encodes a modified amino acid sequence resulting in said polypeptide having reduced RNase H activity, and wherein said polypeptide does not significantly degrade an mRNA template during first strand cDNA synthesis as determined by gel electrophoresis, and wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

23. The polypeptide of claim 22, wherein said nucleotide sequence is an N-MLV nucleotide sequence.

24. The polypeptide of claim 22, wherein said polypeptide does not significantly degrade an mRNA template during cDNA synthesis as shown in

FIG. 5.

25. The polypeptide of claim 22, wherein said polypeptide does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

26. The polypeptide of claim 22, wherein said polypeptide does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

27. The polypeptide of claim 22, wherein said polypeptide does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

28. The polypeptide of claim 22, wherein said polypeptide does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

29. The polypeptide of claim 22, wherein said polypeptide does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

30. An isolated reverse transcriptase having DNA polymerase activity and substantially

reduced RNase H activity, wherein said reverse transcriptase is encoded by a modified nucleotide sequence that encodes a modified amino acid sequence resulting in said reverse transcriptase having substantially reduced RNase H activity, and wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

31. The reverse transcriptase of claim 30, wherein said reverse transcriptase has no detectable RNase H activity.

32. The reverse transcriptase of claim 30, wherein said reverse transcriptase lacks RNase H activity.

33. The reverse transcriptase of claim 30, wherein said reverse transcriptase may be used in the preparation of full length cDNA.

34. The reverse transcriptase of claim 33, wherein said preparation of full length cDNA may be accomplished without significant degradation of an mRNA template.

35. The reverse transcriptase of claim 30, wherein said reverse transcriptase allows the preparation of cDNA without significant degradation of an mRNA template.

36. The reverse transcriptase of claim 30, wherein said reverse transcriptase allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

37. The reverse transcriptase of claim 32, wherein said reverse transcriptase does not significantly degrade an mRNA template during first strand DNA synthesis.

38. The reverse transcriptase of claim 30, wherein said reverse transcriptase does not significantly degrade an mRNA template during a cDNA synthesis reaction as shown in FIG. 5.

39. The reverse transcriptase of claim 30, wherein said reverse transcriptase does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

40. The reverse transcriptase of claim 30, wherein said reverse transcriptase allows an mRNA template to remain intact during a cDNA synthesis reaction as shown in FIG. 5.

41. The reverse transcriptase of claim 30, wherein said reverse transcriptase allows an mRNA template to remain intact during a one minute cDNA synthesis reaction as shown in FIG. 5.

42. The reverse transcriptase of claim 30, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during cDNA synthesis.

43. The reverse transcriptase of claim 30, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during a cDNA synthesis reaction by gel electrophoresis as shown in FIG. 5.

44. The reverse transcriptase of claim 30, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during a one minute reaction as shown in FIG. 5.

45. The reverse transcriptase of claim 30, wherein said reverse transcriptase can synthesize cDNA from an mRNA template.

46. The reverse transcriptase of claim 45, wherein said reverse transcriptase can synthesize full length cDNA.

47. The reverse transcriptase of claim 45, wherein said reverse transcriptase can synthesize cDNA without significant degradation of said mRNA template.

48. The reverse transcriptase of claim 30, wherein said nucleotide sequence is derived from a Moloney Murine Leukemia virus (M-MLV) nucleotide sequence.

49. The reverse transcriptase of claim 30, wherein said nucleotide sequence is derived from a human T-cell leukemia type I (HTLV-1) nucleotide sequence.

50. The reverse transcriptase of claim 30, wherein said nucleotide sequence is derived from a bovine leukemia virus (BLV) nucleotide sequence.

51. The reverse transcriptase of claim 30, wherein said nucleotide sequence is derived from a Rous sarcoma virus (RSV) nucleotide sequence.

52. The reverse transcriptase of claim 30, wherein said nucleotide sequence is derived from a human immunodeficiency virus (HIV) nucleotide sequence.

53. The reverse transcriptase of claim 30, wherein said nucleotide sequence is derived from a retrovirus nucleotide sequence.

54. An isolated reverse transcriptase having DNA polymerase activity and reduced RNase H activity, wherein said reverse transcriptase is encoded by a modified nucleotide sequence that encodes a modified amino acid sequence resulting in said reverse transcriptase having reduced RNase H activity, and wherein said reverse transcriptase does not significantly degrade an mRNA template during first stand cDNA synthesis as determined by gel electrophoresis, and wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

55. The reverse transcriptase of claim 54, wherein said reverse transcriptase does not significantly degrade an mRNA template during a cDNA synthesis reaction as shown in FIG. 5.

56. The reverse transcriptase of claim 54, wherein said reverse transcriptase does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

57. The reverse transcriptase of claim 54, wherein said reverse transcriptase does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

58. The reverse transcriptase of claim 54, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

59. The reverse transcriptase of claim 54, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

60. The reverse transcriptase of claim 54, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

61. The polypeptide of claim 54, wherein said nucleotide sequence is an N-MLV nucleotide sequence.

62. An isolated reverse transcriptase having DNA polymerase activity and substantially reduced RNase H activity, which is obtained by a process comprising

(a) modifying a nucleotide sequence coding for a reverse transcriptase to provide a modified nucleotide sequence that codes for said reverse transcriptase, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents;

(b) transforming a host cell with said modified nucleotide sequence;

(c) culturing the transformed host cell under conditions which produce said reverse transcriptase; and

(d) isolating said reverse transcriptase.

63. The reverse transcriptase of claim 62, wherein said reverse transcriptase has no detectable RNase H activity.

64. The reverse transcriptase of claim 62, wherein said reverse transcriptase lacks RNase H activity.

65. The reverse transcriptase of claim 62, wherein said reverse transcriptase may be used in the preparation of full length cDNA.

66. The reverse transcriptase of claim 65, wherein said preparation of full length cDNA may be accomplished without significant degradation of an mRNA template.

67. The reverse transcriptase of claim 62, herein said reverse transcriptase allows the preparation of cDNA without significant degradation of an mRNA template.

68. The reverse transcriptase of claim 62, wherein said reverse transcriptase allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

69. The reverse transcriptase of claim 62, wherein said reverse transcriptase does not significantly degrade an mRNA template during first strand DNA synthesis.

70. The reverse transcriptase of claim 62, wherein said reverse transcriptase does not significantly degrade an mRNA template during cDNA synthesis as shown in FIG. 5.

71. The reverse transcriptase of claim 62, wherein said reverse transcriptase does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

72. The reverse transcriptase of claim 62, wherein said reverse transcriptase allows an mRNA template to remain intact during a cDNA synthesis reaction as shown in FIG. 5.

73. The reverse transcriptase of claim 62, wherein said reverse transcriptase allows an mRNA template to remain intact during a one minute cDNA synthesis reaction as shown in FIG. 5.

74. The reverse transcriptase of claim 62, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during cDNA synthesis.

75. The reverse transcriptase of claim 62, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template by gel electrophoresis as shown in FIG. 5.

76. The reverse transcriptase of claim 62, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

77. The reverse transcriptase of claim 62, wherein said reverse transcriptase can synthesize cDNA from an mRNA template.

78. The reverse transcriptase of claim 77, wherein said reverse transcriptase can synthesize full length cDNA.

79. The reverse transcriptase of claim 77, wherein said reverse transcriptase can synthesize cDNA without significant degradation of said mRNA template.

80. The reverse transcriptase of claim 62, wherein said reverse transcriptase is a Moloney Murine Leukemia virus (M-MLV) reverse transcriptase.

81. The reverse transcriptase of claim 62, wherein said reverse transcriptase is a human T-cell leukemia type I (HTLV-1) reverse transcriptase.

82. The reverse transcriptase of claim 62, wherein said reverse transcriptase is a bovine leukemia virus (BLV) reverse transcriptase.

83. The reverse transcriptase of claim 62, wherein said reverse transcriptase is a Rous sarcoma virus (RSV) reverse transcriptase.

84. The reverse transcriptase of claim 62, wherein said reverse transcriptase is a human immunodeficiency virus (HIV) reverse transcriptase.

85. The reverse transcriptase of claim 62, wherein said reverse transcriptase is a retrovirus reverse transcriptase.

86. An isolated reverse transcriptase having DNA polymerase activity and reduced RNase

H activity, which is obtained by a process comprising

(a) modifying a nucleotide sequence coding for a reverse transcriptase to

provide a modified nucleotide sequence that codes for said reverse transcriptase, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents;

(b) transforming a host cell with said modified nucleotide sequence;

(c) culturing the transformed host cell under conditions which produce said reverse transcriptase; and

(d) isolating said reverse transcriptase;

wherein said reverse transcriptase does not significantly degrade an mRNA template during first strand cDNA synthesis as determined by gel electrophoresis.

87. The reverse transcriptase of claim 86, wherein said reverse transcriptase does not significantly degrade an mRNA template during a cDNA synthesis reaction as shown in FIG. 5.

88. The reverse transcriptase of claim 86, wherein said reverse transcriptase does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

89. The reverse transcriptase of 86, wherein said reverse transcriptase does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

90. The reverse transcriptase of claim 86, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

91. The reverse transcriptase of claim 86, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

92. The reverse transcriptase of claim 86, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

93. The polypeptide of claim 86, wherein said nucleotide sequence is an M-MLV nucleotide sequence.

94. An isolated recombinantly produced reverse transcriptase having DNA polymerase activity and substantially reduced RNase H activity, wherein said reverse transcriptase is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

95. The reverse transcriptase of claim 94, wherein said reverse transcriptase has no detectable RNase H activity.

96. The reverse transcriptase of claim 94, wherein said reverse transcriptase lacks RNase H activity.

97. The reverse transcriptase of claim 94, wherein said reverse transcriptase may be used in the preparation of full length cDNA.

98. The reverse transcriptase of claim 97, wherein said preparation of full length cDNA may be accomplished without significant degradation of an mRNA template.

99. The reverse transcriptase of claim 94, wherein said reverse transcriptase allows the preparation of cDNA without significant degradation of an mRNA template.

100. The reverse transcriptase of claim 94, wherein said reverse transcriptase does not significantly degrade an mRNA template during a cDNA synthesis reaction as shown in FIG. 5.

101. The reverse transcriptase of claim 94, wherein said reverse transcriptase does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

102. The reverse transcriptase of claim 94, wherein said reverse transcriptase allows an mRNA template to remain intact during a cDNA synthesis reaction as shown in FIG. 5.

103. The reverse transcriptase of claim 94, wherein said reverse transcriptase allows an mRNA template to remain intact during a one minute cDNA synthesis reaction as shown in FIG. 5.

104. The reverse transcriptase of claim 94, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during a cDNA synthesis reaction.

105. The reverse transcriptase of claim 94, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template by gel electrophoresis as shown in FIG. 5.

106. The reverse transcriptase of claim 94, wherein said reduction of RNase H activity is determined by examining the integrity of an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

107. The reverse transcriptase of claim 94, wherein said reverse transcriptase can synthesize cDNA from an mRNA template.

108. The reverse transcriptase of claim 107, wherein said reverse transcriptase can synthesize full length cDNA.

109. The reverse transcriptase of claim 107, wherein said reverse transcriptase can synthesize cDNA without significant degradation of said mRNA template.

110. The reverse transcriptase of claim 94, wherein said reverse transcriptase is a Moloney Murine Leukemia virus (M-MLV) reverse transcriptase.

111. The reverse transcriptase of claim 94, wherein said reverse transcriptase is a human T-cell leukemia type I (HTLV-1) reverse transcriptase.

112. The reverse transcriptase of claim 94, wherein said reverse transcriptase is a bovine leukemia virus (BLV) reverse transcriptase.

113. The reverse transcriptase of claim 94, wherein said reverse transcriptase is a Rous sarcoma virus (RSV) reverse transcriptase.

114. The reverse transcriptase of claim 94, wherein said reverse transcriptase is a human immunodeficiency virus (HIV) reverse transcriptase.

115. The reverse transcriptase of claim 94, wherein said reverse transcriptase is a retrovirus reverse transcriptase.

116. An isolated recombinantly produced reverse transcriptase having DNA polymerase activity and reduced RNase H activity, wherein said reverse transcriptase allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis, and wherein said reverse transcriptase is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

117. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during first strand DNA synthesis.

118. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during first strand DNA synthesis as determined by gel electrophoresis.

119. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during a cDNA synthesis reaction as shown in FIG. 5.

120. The reverse transcriptase of claim 116, wherein said reverse transcriptase does

not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

121. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

122. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

123. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

124. The reverse transcriptase of claim 116, wherein said reverse transcriptase does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

125. The polypeptide of claim 116, wherein said recombinantly produced reverse transcriptase is encoded by an M-MLV nucleotide sequence.

126. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a modified reverse transcriptase nucleotide sequence that encodes an amino acid sequence modified in the region corresponding to amino acids 498-611 of M-MLV reverse transcriptase so that the encoded polypeptide has reduced RNase H activity, and wherein said polypeptide is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

127. The polypeptide of claim 126, wherein said reverse transcriptase nucleotide sequence is selected from the group consisting of M-MLV, HTLV-1, BLV, and HIV reverse transcriptase nucleotide sequences.

128. The polypeptide of claim 126, wherein said nucleotide sequence is derived from a retrovirus nucleotide sequence.

129. The polypeptide of claim 126, wherein said region corresponds to amino acids 503-611 of the M-MLV reverse transcriptase.

130. The polypeptide of claim 126, wherein said reverse transcriptase nucleotide sequence is a M-MLV nucleotide sequence.

131. The polypeptide of claim 126, wherein said polypeptide allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

132. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during first strand cDNA synthesis.

133. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during cDNA synthesis as shown in FIG. 5.

134. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

135. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

136. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

137. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

138. The polypeptide of claim 126, wherein said polypeptide does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

139. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a reverse transcriptase nucleotide sequence that has been modified so that the encoded polypeptide has reduced RNase H activity, wherein said polypeptide is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents, and wherein said polypeptide may be used for the preparation of cDNA of which at least 34% is full length.

140. The polypeptide of claim 139, wherein said nucleotide sequence is derived from an organism selected from the group consisting of M-MLV, HTLV-1, BLV, and HIV.

141. The polypeptide of claim 139, wherein said nucleotide sequence is derived from a retrovirus nucleotide sequence.

142. The polypeptide of claim 139, wherein said reverse transcriptase nucleotide sequence is a M-MLV nucleotide sequence.

143. The polypeptide of claim 139, wherein said polypeptide allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

144. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during first strand cDNA synthesis.

145. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during cDNA synthesis as shown in FIG. 5.

146. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

147. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

148. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

149. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

150. The polypeptide of claim 139, wherein said polypeptide does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

151. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a reverse transcriptase nucleotide sequence that has been modified so that the encoded polypeptide has reduced RNase H activity, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents, wherein said polypeptide may be used for the preparation of full length cDNA, and wherein the amount of full length cDNA is at least 42% greater than the amount of full length cDNA obtained by a polypeptide encoded by said nucleotide sequence that has not been so modified.

152. The polypeptide of claim 151, wherein said nucleotide sequence is derived from an organism selected from the group consisting of M-MLV, HTLV-1, BLV, and HIV.

153. The polypeptide of claim 151, wherein said nucleotide sequence is derived from a retrovirus nucleotide sequence.

154. The polypeptide of claim 151, wherein said reverse transcriptase nucleotide sequence is a M-MLV nucleotide sequence.

155. The polypeptide of claim 151, wherein said polypeptide allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

156. The polypeptide of claim 151, wherein said polypeptide does not significantly

degrade an mRNA template during first strand cDNA synthesis.

157. The polypeptide of claim 151, wherein said polypeptide does not significantly degrade an mRNA template during cDNA synthesis as shown in FIG. 5.

158. The polypeptide of claim 151, wherein said polypeptide does not significantly degrade an mRNA template during a one minute cDNA synthesis reaction as shown in FIG. 5.

159. The polypeptide of claim 151, wherein said polypeptide does not significantly degrade an mRNA template during a five minute cDNA synthesis reaction as shown in FIG. 5.

160. The polypeptide of claim 151, wherein said polypeptide does not significantly degrade an mRNA template during a 10 minute cDNA synthesis reaction as shown in FIG. 5.

161. The polypeptide of claim 151, wherein said polypeptide does not significantly degrade an mRNA template during a 30 minute cDNA synthesis reaction as shown in FIG. 5.

162. The polypeptide of claim 151, wherein said polypeptide does not significantly degrade an mRNA template during a 60 minute cDNA synthesis reaction as shown in FIG. 5.

163. An isolated polypeptide having DNA polymerase activity and substantially reduced RNase H activity, wherein said polypeptide is encoded by a modified reverse transcriptase nucleotide sequence that encodes an amino acid sequence modified within the RNase H domain so that the encoded polypeptide has substantially reduced RNase H activity.

164. The polypeptide of claim 163, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

165. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a modified reverse transcriptase nucleotide sequence that encodes an amino acid sequence modified within the RNase H domain so that the encoded polypeptide has reduced RNase H activity, and wherein said polypeptide does not significantly degrade an mRNA template during first strand cDNA synthesis as determined by gel electrophoresis.

166. The polypeptide of claim 165, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

167. An isolated reverse transcriptase having DNA polymerase activity and substantially reduced RNase H activity, wherein said reverse transcriptase is encoded by a modified nucleotide sequence that encodes an amino acid sequence modified within the RNase H domain so that the encoded reverse transcriptase has substantially reduced RNase H activity.

168. The reverse transcriptase of claim 167, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

169. An isolated reverse transcriptase having DNA polymerase activity and reduced RNase H activity, wherein said reverse transcriptase is encoded by a modified nucleotide sequence that encodes an amino acid sequence modified within the RNase H domain so that the encoded reverse transcriptase has reduced RNase H activity, and wherein said reverse transcriptase does not significantly degrade an mRNA template during first stand cDNA synthesis as determined by gel electrophoresis.

170. The reverse transcriptase of claim 169, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

171. An isolated reverse transcriptase having DNA polymerase activity and substantially reduced RNase H activity, which is obtained by a process comprising

(a) modifying within the RNase H domain of a nucleotide sequence coding for a reverse transcriptase to provide a modified nucleotide sequence that codes for said reverse

transcriptase;

(b) transforming a host cell with said modified nucleotide sequence;

(c) culturing the transformed host cell under conditions which produce said reverse transcriptase; and

(d) isolating said reverse transcriptase.

172. The reverse transcriptase of claim 171, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

173. An isolated reverse transcriptase having DNA polymerase activity and reduced RNase H activity, which is obtained by a process comprising

(a) modifying within the RNase H domain of a nucleotide sequence coding for a reverse transcriptase to provide a modified nucleotide sequence that codes for said reverse transcriptase;

(b) transforming a host cell with said modified nucleotide sequence;

(c) culturing the transformed host cell under conditions which produce said reverse transcriptase; and

(d) isolating said reverse transcriptase;

wherein said reverse transcriptase does not significantly degrade am mRNA template during first strand cDNA synthesis as determined by gel electrophoresis.

174. The reverse transcriptase of claim 173, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

175. An isolated recombinantly produced reverse transcriptase having DNA polymerase activity and substantially reduced RNase H activity, wherein said reverse transcriptase has been modified within the RNase H domain to give substantially reduced RNase H activity.

176. The reverse transcriptase of claim 175, wherein said reverse transcriptase is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

177. An isolated recombinantly produced reverse transcriptase having DNA polymerase activity and reduced RNase H activity, wherein said reverse transcriptase has been modified within the RNase H domain to give substantially reduced RNase H activity, and wherein said reverse transcriptase allows preparation of cDNA without significant degradation of an mRNA template as determined by gel electrophoresis.

178. The reverse transcriptase of claim 177, wherein said reverse transcriptase is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

179. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a reverse transcriptase nucleotide sequence that has been modified within the RNase H domain so that the encoded polypeptide has reduced RNase H activity, and wherein said polypeptide may be used for the preparation of cDNA of which at least 34% is full length.

180. The polypeptide of claim 179, wherein said polypeptide is encoded by a nucleotide sequence derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

181. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a reverse transcriptase nucleotide sequence that has been modified within the RNase H domain so that the encoded polypeptide has reduced RNase H activity, wherein said polypeptide may be used for the preparation of full length cDNA, and wherein the amount of full length cDNA is at least 42% greater than the amount of full length cDNA obtained by a polypeptide encoded by

said nucleotide sequence that has not been so modified.

182. The polypeptide of claim 181, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

183. An isolated polypeptide having DNA polymerase activity and reduced RNase H activity, wherein said polypeptide is encoded by a modified reverse transcriptase nucleotide sequence that encodes an amino acid sequence modified within the RNase H domain corresponding to amino acids 498-611 of M-MLV reverse transcriptase so that the encoded polypeptide has reduced RNase H activity.

184. The polypeptide of claim 183, wherein said nucleotide sequence is derived from an organism selected from the group consisting of a retrovirus, yeast, Neurospora, Drosophila, primates and rodents.

185. An isolated DNA molecule comprising a nucleotide sequence encoding the polypeptide or reverse transcriptase of any one of claims 1, 22, 25, 30, 54, 56, 62, 86, 94, 116, 120, 126-134, 139, 151, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181 and 183.

186. A vector comprising the DNA molecule of claim 185.

187. A host cell comprising the DNA molecule of claim 189.

188. A host cell comprising the vector of claim 155.

189. A method for preparing a DNA molecule, said method comprising:

mixing an mRNA template with the polypeptide or reverse transcriptase of any one of claims 1, 22, 25, 30, 54, 56, 62, 86, 94, 116, 120, 126-134, 139, 151, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181 and 183; and

incubating said mixture under conditions sufficient to make a first DNA molecule complementary to said mRNA template.

190. A method for producing a polypeptide or reverse transcriptase having DNA polymerase activity and reduced RNase H activity, said method comprising:

culturing the host cell of claim 187 under conditions sufficient to produce said polypeptide or reverse transcriptase; and

isolating said polypeptide or reverse transcriptase.

191. A method for producing a polypeptide or reverse transcriptase having DNA polymerase activity and reduced RNase H activity, said method comprising:

culturing the host cell of claim 188 under conditions sufficient to produce said polypeptide or reverse transcriptase; and

isolating said polypeptide or reverse transcriptase.

192. The method of claim 189, wherein said first DNA molecule is full length cDNA.

193. The method of claim 189, further comprising incubating said first DNA molecule under conditions sufficient to make a second DNA molecule complementary to said first DNA molecule.

194. The method of claim 193, wherein said first and second DNA molecule form a double stranded DNA molecule.

195. A kit for the preparation of cDNA, said kit comprising a container containing the polypeptide or reverse transcriptase of any one of claims 1, 22, 25, 30, 54, 56, 62, 86, 94, 116, 120, 126-134, 139, 151, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, and 183.

196. The kit of claim 195, said kit further comprising one or more additional containers selected from the group consisting of:

a container containing one or more nucleoside triphosphates;

a container containing an oligo (dT) primer; and

a container containing a buffer suitable for use in making cDNA.

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L1: Entry 1 of 1 File: USPT May 21, 1991

US-PAT-NO: 5017492
DOCUMENT-IDENTIFIER: US 5017492 A

TITLE: Reverse transcriptase and method for its production

DATE-ISSUED: May 21, 1991

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
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US-CL-CURRENT: 435/252.3; 435/252.33, 435/320.1

CLAIMS:

We claim:

1. A plasmid carrying genetic material derived from murine leukemia virus, wherein said genetic material encodes a protein truncated three amino acids or more from the carboxy terminus of native murine leukemia virus reverse transcriptase, wherein said protein exhibits reverse transcriptase activity with a half-life of 26-35 minutes.

2. A plasmid carrying genetic material derived from murine leukemia virus, wherein said genetic material encodes a protein truncated three amino acids or more from the carboxy terminus of native murine leukemia virus reverse transcriptase, wherein said protein contains an .alpha.-helix of 18 amino acids adjacent to the carboxy end of said protein.

3. A bacterial cell containing the plasmid of claim 1 or 2.

4. Plasmid pRT601.

5. E. coli strain N4830 containing plasmid pRT601.